E2 subunit marker vaccines reduce transmission of classical swine fever virus sufficiently to halt epidemics

D. Klinkenberg, A. Bouma, G. Floegel-Niesmann, M.C.M. de Jong

Submitted to Preventive Veterinary Medicine
Abstract

This paper presents a more comprehensive analysis of a previously published Classical Swine Fever Virus (CSFV) transmission experiment. In the experiment, two E2 subunit marker vaccines, one from Bayer, Germany (A) and one from Intervet, the Netherlands (B), had been tested to establish the time interval between vaccination and protection against horizontal virus transmission. The original analysis led to the conclusion that the vaccines mutually differed and that they reduced transmission as from 14 days after vaccination with either vaccine. In the analysis, however, virus transmission was not quantified. Therefore, it did not allow for extrapolation of the results to larger populations. Because the expected vaccine effectiveness on population level will be important for the decision to use emergency vaccination during a CSFV epidemic or not, a more comprehensive analysis was deemed necessary. The new analysis tested the vaccines in their ability to decrease the basic reproduction ratio $R_0$, which is defined as the average number of susceptible animals that will be infected by one infectious animal in a susceptible population. An important property of $R_0$ is its threshold value 1: if $R_0 < 1$, no epidemics can occur. Two methods for analysis were used. The first method, the final size method, determined for each tested vaccination-challenge interval whether $R_0$ was significantly reduced. It appeared that $R_0$ was significantly reduced after 14 days with vaccine A and after 21 days with vaccine B. The second method, the entire course method, was used to determine the time the vaccines needed to bring $R_0$ below 1, and to determine $R_0$ in an unvaccinated and in a vaccinated population. With both vaccines $R_0$ was significantly smaller than 1 after three weeks. In an unvaccinated population, $R_0$ was estimated at 9.9. Vaccine A let $R_0$ decrease to 0.047, whereas vaccine B reduced $R_0$ to 0.41. Although $R_0$ with vaccine A significantly differed from $R_0$ with vaccine B, stochastic simulations showed that either vaccine will limit most CSFV outbreaks in a pig unit of 100 pigs to only one infected pig (95% CI with vaccine A: 1-2; with vaccine B: 1-6).

3.1. Introduction

Classical swine fever (CSF, syn. hog cholera) is an infectious disease of swine, caused by the CSF virus (CSFV) (for more general information, cf. Taylor, 1995; Van Oirschot, 1992). Introduction of CSFV into a susceptible pig population can cause large epidemics which can affect many pig farms and become very costly, as seen in the Dutch CSFV epidemic of 1997/1998 (Elbers et al., 1999; Meuwissen et
al., 1999). Vaccination might reduce size and costs of CSFV epidemics, but the legislation of the European Union (EU) does not allow prophylactic vaccination against CSF since 1980 (Anonymous, 1980; Anonymous, 2001). Although use of the C-strain vaccine has been shown to accomplish CSFV eradication in the 1980s (Terpstra and Wensvoort, 1987) and the EU does allow emergency vaccinations during epidemics (Anonymous, 2001), they have never been applied because of the resulting export restrictions (Moennig, 2000).

The reason for export restrictions is that vaccinated animals cannot be distinguished from infected animals, and therefore trade with vaccinated animals is a risk. Because of this, marker vaccines have been developed, based on the E2-glycoprotein of the virus (Hulst et al., 1993; König et al., 1995). Vaccination with an E2 marker vaccine induces antibodies against only the E2 protein of the virus. An ELISA was designed to detect antibodies against the E2 protein of the virus and can be used as a discriminating test (Moormann et al., 2000; van Rijn et al., 1999).

Applying emergency vaccination with an E2 vaccine will only be useful if the vaccine is sufficiently capable of reducing the size and costs of an epidemic. Therefore, the EU financed three marker vaccine experiments to test the efficacy of two E2 marker vaccines, BAYOVAC CSF marker (Bayer, Germany) and PORCILIS PESTI (Intervet, The Netherlands) (Depner et al., 2001; Floegel-Niesmann, 2001; Uttenthal et al., 2001). One of the experiments was to establish the effect on horizontal virus transmission (Uttenthal et al., 2001). The main conclusions of this experiment were that there is a difference in virus transmission between the vaccines, and that vaccination reduces virus shedding and spreading as from 14 days after vaccination.

A problem with the analysis of Uttenthal et al. (2001) is that it does not answer the question whether the vaccines sufficiently reduce CSFV transmission to prevent major outbreaks on pig farms. Since the conclusions were based on the Fisher’s exact test and on qualitative data analysis, it is impossible to predict the effect of vaccination on transmission, if the virus would enter a vaccinated pig herd. Better predictions can be made if transmission of an infectious agent is quantified by the basic reproduction ratio, $R_0$: the average number of individuals that is infected by one infectious individual in an entirely susceptible population (cf. Anderson and May, 1991; Diekmann and Heesterbeek, 2000). A nice property of $R_0$ is its threshold value: if $R_0$ is smaller than 1, only minor outbreaks can occur and only if $R_0$ is larger than 1, the virus can spread and major outbreaks can occur. Because of this threshold property, it is very useful to analyse transmission experiments by use of $R_0$, either by testing the null-hypothesis that $R_0$ is equal in two groups, or by estimating $R_0$ in the different groups and testing whether it is smaller (or larger) than 1.
This paper presents an analysis of the EU horizontal-transmission trials with respect to $R_0$. The objective of the analysis was to determine whether the vaccines are able to reduce $R_0$ to a value smaller than 1, and to determine the time from vaccination until $R_0$ becomes smaller than 1. Since the trials were conducted in five different laboratories, a meta-analysis of all trials was conducted. A meta-analysis was considered appropriate, since many important aspects of the trials were the same in all laboratories: the animals’ age and health status (no other infections), the vaccine batches, the CSFV challenge strain, and the diagnostic tests used to assess CSFV infection.

In our analysis, we used two methods, both based on a mathematical model that describes the transmission of infectious agents, the SIR model (Bailey, 1975). With the first method, the final size method (Kroese and De Jong, 2001), it was tested whether the $R_0$ of the vaccine groups was significantly reduced compared to the control group. With the second method, the entire course method (Chapter 2), two epidemiological parameters were estimated with time after vaccination, viz. the transmission parameter $\beta$ and the recovery parameter $\alpha$. The estimates for $\beta$ and $\alpha$ were used to determine the time lag between vaccination and the situation where $R_0 < 1$, and to estimate $R_0$ with and without vaccination.

3.2. Materials and Methods

3.2.1. Experimental procedures

The experimental procedures of the transmission trials are described in detail by Uttenthal et al. (2001). Here we give a summary, where we focus on the aspects that are important for interpretation of the results with respect to reduction of virus transmission.

3.2.1.1. Experimental design

A total of 190 conventional weaner pigs of 5-6 weeks of age had been used in 19 transmission trials with ten pigs per trial. The 19 trials had been divided into nine treatment groups. Four treatment groups consisting of two trials had been vaccinated with vaccine A (BAYOVAC® CSF marker E2/98/B001, Bayer, Germany). Another four treatment groups of two trials had been vaccinated with vaccine B (PORCILIS® PESTI January 1999, Intervet, The Netherlands). The ninth treatment group of three trials was used as the unvaccinated control group.
All trials had started by challenging five out of ten animals with a CSFV field strain isolated in Germany (Paderborn). Four different time intervals between vaccination and challenge were studied in the four treatment groups per vaccine. The intervals were 7 days (groups A7 and B7), 10 days (groups A10 and B10), 14 days (groups A14 and B14), and 21 days (groups A21 and B21). The transmission trials of the unvaccinated control group (group C) had started at day 0. After challenge, every two to eight days the animals had been sampled for determination of viraemia and antibodies against the E\text{\textsuperscript{mp}}-epitope of the CSFV. Body temperature had been measured daily. At the end of the trials, tissue samples had been taken for virus detection.

3.2.1.2. Organisation of the experiment

Four national swine fever laboratories in Italy, Spain, France, and Denmark and the EU reference laboratory for CSF in Germany had been involved in carrying out the transmission trials. Besides the unavoidable differences between the laboratories with regard to management and handling of the animals, more specific differences are listed in Table 3.1. Because of the differences, Uttenthal et al. (2001) had decided only to compare groups within laboratories. Because of the major similarities between the trials in the different laboratories, we considered a meta-analysis of all trials appropriate. The meta-analysis made a more comprehensive analysis possible.

3.2.2. Statistical analysis

3.2.2.1. Transmission trials and $R_0$

Transmission trials start with only five infectious pigs and five susceptible\(^1\) contact pigs, whereas the basic reproduction ratio $R_0$ is defined in an infinite population (cf. Anderson and May, 1991; Diekmann and Heesterbeek, 2000). The stochastic SIR model (Bailey, 1975) can be used to translate the data of transmission trials to $R_0$. ‘SIR’ refers to the assumption that each animal is a member of one of the classes S (susceptible), I (infectious), or R (removed, i.e. dead or immune). The animals can proceed from the S class to the I class by infection, due to the presence of infectious animals. Each infectious animal infects susceptible animals with rate $\beta s/N$, where $s$

\(^1\) In this paper, ‘susceptible’ means ‘not yet infected’, so vaccinated animals can also be susceptible.
E2 vaccines reduce transmission

is the actual number of susceptible pigs, \( N \) the total number of present pigs, and \( \beta \) is the transmission parameter. Animals in the I class can move to the R class by recovery or death, which happens at rate \( \alpha \), the recovery parameter. The average length of the infectious period is \( 1/\alpha \).

From the SIR model, \( R_0 \) can be determined by regarding an infinite susceptible population, in which \( s \approx N \). This results in an infection rate of \( \beta \) and a basic reproduction ratio, which is the average number of new infections per day times the average length of the infectious period, of \( R_0 = \beta/\alpha \) (Diekmann and Heesterbeek, 2000).

### 3.2.2.2. The final size method

The final size method (De Jong and Kimman, 1994; Kroese and De Jong, 2001) was used to test whether the \( R_0 \) in the eight different vaccine groups was significantly lower than the \( R_0 \) in the control group C. The null-hypothesis to test a vaccine group V (e.g. group A7) against the control group C was \( H_0: R_{0,V} = R_{0,C} \). The method was used, because it was perfectly fit for the design of the trials and because it was used in previous transmission experiments (Bouma et al., 2000; Chapter 4). Interpretation of the results is however not straightforward, because the method assumes a constant \( R_0 \) in each trial, which was probably unrealistic for the vaccination-challenge intervals of 7 and 10 days, and maybe for 14 and 21 days as well. In the discussion section, the interpretation will be addressed.

### Table 3.1. Comparison of the experimental procedures in the participating laboratories

<table>
<thead>
<tr>
<th>Country</th>
<th>Groups</th>
<th>Challenge route</th>
<th>dose (TCID50)</th>
<th>Irregularities</th>
</tr>
</thead>
<tbody>
<tr>
<td>France</td>
<td>A14, A21, B14, B21, C</td>
<td>Intranasal</td>
<td>10^4.4</td>
<td>some pigs were BVD^a</td>
</tr>
<tr>
<td>Denmark</td>
<td>A14, A21, B14, B21, C</td>
<td>Oral</td>
<td>10^3.4</td>
<td>All trials in one isolation unit</td>
</tr>
<tr>
<td>Spain</td>
<td>A7, A10, B7, B10</td>
<td>Intranasal</td>
<td>10^5</td>
<td></td>
</tr>
<tr>
<td>Italy</td>
<td>A7, A10, B7, B10</td>
<td>Intranasal</td>
<td>10^5</td>
<td></td>
</tr>
<tr>
<td>Germany</td>
<td>C</td>
<td>Oronasal</td>
<td>10^3</td>
<td></td>
</tr>
</tbody>
</table>

^a At time of challenge, 20% of the French pigs was still BVD. Before vaccination, BVD pigs had been equally distributed among the five trials.
Chapter 3

The data needed for the final size method were the initial state and the final size of each transmission trial. The initial state consists of the number of challenged (infectious) animals \(i_0\) and the number of contact (susceptible) animals \(s_0\) at the start of the transmission trial. The final size is the number of contact animals that was still susceptible by the end of the trial, \(s_t\). Therefore, for each contact animal it had to be determined whether it had been infected during the trial. As in Uttenthal et al. (2001), a contact animal was defined infected if it had been positive at least once in the E\(^{\text{imm}}\) ELISA, virus isolation from blood samples, or virus detection from tissue samples.

The test statistic for the final size method was the difference in the number of contact infections between the vaccine and control group. The number of contact infections was \(s_t - s_0\) for each trial. The calculated \(P\)-value is the probability that the difference (or larger) is observed given \(H_0\). A \(P\)-value \(< 0.05\) was regarded statistically significant.

3.2.2.3. The entire course method

The entire course method was used to estimate the parameters \(\beta\) and \(\alpha\) of the SIR model in the second, third, and fourth week after vaccination, and from the fifth week onwards. Although it is a complicated method, which needs more data and assumptions than the final size method, it can cope with the fact that the parameters change with time. Moreover, the estimates of \(\beta\) and \(\alpha\) can be used to estimate \(R_0\). Because a changing \(R_0\) due to a changing \(\beta\) or \(\alpha\) is hard to interpret, \(R_0\) was only determined for situations with constant \(\beta\) and \(\alpha\), which was in the unvaccinated group C and three weeks after vaccination in groups A and B.

The data needed for the entire course method are exact reconstructions of all transmission trials, that is, for each animal the time of infection, the period of infectiousness, and the time of death were needed. Exact reconstructions could not be made directly from the data, because they were not precise enough. Therefore, 100 possible exact reconstructions were drawn using distributions of the infection times, and of the start and end of the infectious periods for each animal. The distributions were obtained from the viraemia and serology data.

In short, the entire course method consisted of three successive steps. The first was the interpretation of the viraemia and serology data to obtain distributions for the time of infection and the start and end of the infectious period of each animal. The second was the drawing of 100 exact reconstructions with these distributions. The third was the estimation of \(\beta\) and \(\alpha\) from these exact reconstructions.
3.2.2.4. Entire course method step 1: data interpretation

To determine the start and end of the infectious period, the assumption was made that infectiousness had coincided with viraemia, as had been assumed in previous analyses (Chapter 2; Chapter 4; Laevens et al., 1998; Laevens et al., 1999). In all trials, blood samples had been taken in time intervals of two to eight days for virus isolation. It was assumed that the start of the infectious period of each animal was uniformly distributed within the time interval before the first positive sample. The end of the infectious period was either uniformly distributed within the time interval after the last positive sample or it was determined by death of the animal when it had still been virus-positive by then.

The time of infection could be reconstructed in one of four possibilities. First, if the animal had been challenged, the time of infection was equal to the time of challenge. Second, if the animal had not shown viraemia and had not been seropositive in the E\textsuperscript{iso} ELISA (the discriminatory ELISA), it had not been infected at all, so there was no infection time. Third, if the animal had shown viraemia, it was assumed that the infection time had been one latent period before the start of the infectious period. The latent period is the interval between becoming infected and infectious. It was determined from the challenged animals in the same laboratory as the mode of their latent periods. Fourth, if the animal had not shown viraemia, but had been seropositive, the interval of seroconversion was determined and the moment of seroconversion was assumed to be uniformly distributed within the interval. Subsequently, a distribution for the time between infection and first seropositivity was needed. It was assumed that this was a lognormal distribution of which the parameters — $\mu_A$ and $\sigma_A^2$ for the test with vaccine A, or $\mu_B$ and $\sigma_B^2$ for the test with vaccine B — were determined from the data of the challenged animals (see Appendix 3A).

For some situations, the interpretation of the viraemia and serology data as described could not be followed. Then, exceptions were necessary and the data had to be interpreted case by case. The exceptions are given in the Tables with the Results section (Tables 3.2 – 3.5).

3.2.2.5. Entire course method step 2: exact reconstructions

Each transmission trial was reconstructed 100 times in five steps:
1. If the animal had been infectious, the start of the infectious period was drawn from the interval of first viraemia.
2. If the animal had been infectious, the end of the infectious period was drawn from the interval of last viraemia or it was set at the day of death.
Chapter 3

3. If the animal had been infected, the time of infection was determined. For challenged pigs, it was the challenge day. For contact pigs, it was the length of the latent period subtracted from the start of the infectious period, or it was drawn from the appropriate distribution by using the interval of first seropositivity.

4. The day of death of the animal. This was used to define the population size in the trial at each time.

5. Finally, it was checked whether each drawn infection time coincided with at least one animal that was infectious. If that was not the case, the process was restarted at step 1 until a realistic reconstruction was obtained.

3.2.2.6. Entire course method step 3: parameter estimation

The reconstructions were divided into three sets, all vaccine A trials, all vaccine B trials and all group C trials. The group C trials were used to estimate $\beta$, $\alpha$, and $R_0$ in an unvaccinated population. Each set of vaccine trials was used to estimate $\beta$ and $\alpha$ in the second, third, and fourth week after vaccination, and from the fifth week onwards. Then it was tested whether $\beta$ and $\alpha$ were significantly different from week to week. If $\beta$ (or $\alpha$) did not differ significantly in successive weeks, the weeks were pooled and $\beta$ (or $\alpha$) was re-estimated for the longer period of two or more weeks. The $\beta$ and $\alpha$ from the periods the longest after vaccination, which include the fifth week onwards, were used to estimate $R_0$ in a vaccinated population.

Before estimation was possible, assumptions were necessary on how $\beta$ and $\alpha$ depend on time after vaccination. In the most complex situation, the recovery and infection rates of an infectious animal at time $t_{\text{now}}$ depend on (1) the time that the animal was infected $t_{\text{inf}}$ and (2) $t_{\text{now}}$. Since the recovery rate reflects the ability of the animal’s immune system to clear the virus, it highly depends on the state of the immune system at the time of infection. Hence, $\alpha$ was assumed to depend only on $t_{\text{inf}}$. The infection rate of an animal reflects the animal's immune response too, but it also depends on the susceptibility of the yet uninfected animals. Therefore, $\beta$ would depend on both $t_{\text{inf}}$ as $t_{\text{now}}$, which would create a model with too many different $\beta$'s in relation to the available data. Because the immune response is already covered by $\alpha$, it was assumed that $\beta$ only depends on $t_{\text{now}}$. In short, the $\alpha$ of a certain week is the rate by which animals that are infected in that week will recover and the $\beta$ of a certain week is, multiplied by $s/N$, the rate by which infectious animals cause new infections within that very week.

Both $\beta$ and $\alpha$ were estimated with a generalised linear model (GLM). The method to estimate $\alpha$ had been used before in Chapter 2, although it had to be
E2 vaccines reduce transmission

adjusted because of the use of 100 reconstructions. A similar method was used to estimate $\beta$ (see Appendix 3B). The results consisted of one estimate for $\log\beta$ with variance and one estimate for $\log\alpha$ with variance, for the week or weeks of interest. These were used to test whether the parameters were different in the successive weeks and for construction of confidence intervals. The estimates and variances were also used to estimate $\log R_0$ and its variance:

$$\log R_0 = \log \beta - \log \alpha$$
$$\text{var}(\log R_0) = \text{var}(\log \beta) + \text{var}(\log \alpha),$$

from which an estimate and a 95% confidence interval for $R_0$ were derived. Note that the $\text{cov}(\log \beta, \log \alpha)$ is assumed to be 0. Because $\text{cov}(\log \beta, \log \alpha)$ will not be negative, – a larger $\alpha$ will lead to shorter infectious periods, which has to be compensated by a larger $\beta$ to obtain the same number of contact infections – $\text{var}(\log R_0)$ will be overestimated. Overestimation results in too wide confidence intervals for $R_0$.

3.2.3. Vaccination and outbreak size

As mentioned before, it is important to know if the vaccines are able to sufficiently reduce the outbreak size. Therefore, simulations were conducted in which one pig in a group of 100 pigs was infected by CSFV and virus transmission occurred according to the SIR model with the estimated parameters $\beta$ and $\alpha$. The parameters were allowed to change if the estimated values changed per week after vaccination.

A comparison was made between an unvaccinated group and groups completely protected by either vaccine A or B. In addition it was examined to what extent the outbreak sizes will be reduced if virus would enter a herd only 7 days after vaccination, when the vaccines are not yet completely protective.

The simulations were carried out in Mathematica® (Wolfram, 1999). For each of the five different situations, 1000 simulations were performed. The situations were compared with the number of pigs that were ultimately infected during the simulation, which is the final size of the outbreak.

3.3. Results

3.3.1. The final size method
Chapter 3

The data of all transmission trials are listed in Table 3.2 and were used for the test with \( H_0: R_{0,V} = R_{0,C} \). The resulting \( P \)-values are shown in Table 3.2 as well. CSFV transmission was significantly reduced in groups A7, A14, and A21, however not in group A10. Transmission was also significantly reduced in group B21.

### Table 3.2. The final size method: data and results.

<table>
<thead>
<tr>
<th>Trial</th>
<th>( s_0^a )</th>
<th>( i_0^b )</th>
<th>( s_1^c )</th>
<th># c.i. (^d)</th>
<th>( P^e )</th>
</tr>
</thead>
<tbody>
<tr>
<td>C - Germany</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C - Denmark f</td>
<td>7</td>
<td>3</td>
<td>0</td>
<td>18</td>
<td>-</td>
</tr>
<tr>
<td>C - France f</td>
<td>6</td>
<td>4</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A7 - Spain g</td>
<td>4</td>
<td>6</td>
<td>0</td>
<td></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>A7 - Italy</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>A10 - Spain h</td>
<td>4</td>
<td>5</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A10 - Italy</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>7</td>
<td>0.11</td>
</tr>
<tr>
<td>A14 - Denmark</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>A14 - France</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>A21 - Denmark</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>A21 - France</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>B7 - Spain</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td></td>
<td>0.11</td>
</tr>
<tr>
<td>B7 - Italy b</td>
<td>4</td>
<td>5</td>
<td>2</td>
<td>7</td>
<td>0.23</td>
</tr>
<tr>
<td>B10 - Spain</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B10 - Italy</td>
<td>5</td>
<td>5</td>
<td>1</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>B14 - Denmark</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B14 - France</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>B21 - Denmark</td>
<td>5</td>
<td>5</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B21 - France</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

\(^a\) The number of susceptible animals at the start of the trial  
\(^b\) The number of infected animals at the start of the trial  
\(^c\) The number of susceptible animals at the end of the trial  
\(^d\) The total number of contact infection in the treatment group  
\(^e\) The \( P \)-value of the final size test for testing against group C  
\(^f\) Because in these trials one and two pigs were only viraemic at the time of the other contact pigs, it was assumed that the inoculation had been unsuccessful and the pigs were regarded as contact pigs  
\(^g\) In this trial, one animal was viraemic at day 11, just like the challenged animals, and has therefore been treated as a challenged animal  
\(^h\) In these trials, one pig had died before the challenge day

The data of all transmission trials are listed in Table 3.2 and were used for the test with \( H_0: R_{0,V} = R_{0,C} \). The resulting \( P \)-values are shown in Table 3.2 as well. CSFV transmission was significantly reduced in groups A7, A14, and A21, however not in group A10. Transmission was also significantly reduced in group B21.
3.3.2. The entire course method

First, the parameters $\mu$ and $\sigma^2$ of the distribution of the challenge-seropositivity interval were determined for both E$^{m}$ ELISAs. The challenged animals were found to seroconvert by ELISA A in the following time intervals after challenge:

\[
8 – 10 (3\times), 9 – 11 (8\times), 10 – 12 (7\times), 12 – 14 (2\times), 12 – 15, 14 – 16, 14 – 21, 15 – 18, 21 – 25, 21 – 28, 28 – 35 (4\times), 35 – 42 (5\times),
\]

from which $\mu_A$ was estimated at 2.73 and $\sigma^2_A$ at 0.279. With the vaccine B ELISA, the time intervals of seroconversion were found to be

\[
6 – 8, 8 – 10 (9\times), 9 – 12, 10 – 12 (12\times), 12 – 14 (7\times), 12 – 15 (3\times), 12 – 17 (4\times), 14 – 21 (2\times), 17 – 23 (3\times), 18 – 22, 21 – 28,
\]

from which $\mu_B$ was estimated at 2.49 and $\sigma^2_B$ at 0.0635.

The data for the entire course method are listed in Table 3.3 for all groups A, in Table 3.4 for all groups B, and in Table 3.5 for group C. Table 3.6 lists the estimates of $\beta$ and $\alpha$ from the vaccine-A trials and from the group-C trials and Table 3.7 lists the estimates from the vaccine-B trials and the group-C trials.

The $\beta$ estimated from group C was 0.65 (95% CI: 0.40 – 1.1), whereas the $\alpha$ was estimated at 0.065 (95% CI: 0.045 – 0.096). The resulting $R_0$ in unvaccinated pigs was 9.9 (95% CI: 5.3 – 18).

For vaccine A (Table 3.6), the transmission parameter $\beta$ did not differ significantly between time intervals. Consequently, all data were pooled and $\beta$ was estimated for the whole period after vaccination, which led to an estimate of 0.29 (95% CI: 0.17 – 0.48), which was significantly lower than the $\beta$ of group C. Recovery parameter $\alpha$ was estimated in the same time intervals as $\beta$. The $\alpha$ appeared to increase weekly until at least the fourth week after vaccination. From the fifth week onwards, only one data point was available, with $T_i = 0$. Therefore, $\alpha$ was re-estimated for the whole period from 21 days after vaccination at 6.1 (95% CI: 2.4 – 16), which was significantly higher than the $\alpha$ of group C. As explained in section 2.2.3, the basic reproduction ratio $R_0$ was only estimated with constant $\beta$ and $\alpha$, which was 21 days after vaccination: $R_0$ was 0.047 (95% CI: 0.016 – 0.14). Thus, vaccine A significantly reduced $R_0$, which reached a value significantly smaller than 1.
Table 3.3. Reconstruction of the transmission trials of group A.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Time of infection(^b) (days(^a))</th>
<th>Start of infectious period(^c) (days(^a))</th>
<th>End of infectious period(^d) (days(^a))</th>
<th>Trial</th>
<th>Time of infection(^b) (days(^a))</th>
<th>Start of infectious period(^c) (days(^a))</th>
<th>End of infectious period(^d) (days(^a))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>9 – 11</td>
<td>24</td>
<td>14</td>
<td>14</td>
<td>18 – 20</td>
<td>20 – 22</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>9 – 11</td>
<td>15 – 17</td>
<td>14</td>
<td>14</td>
<td>18 – 20</td>
<td>20 – 22</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>9 – 11</td>
<td>17 – 19</td>
<td>14</td>
<td>14</td>
<td>18 – 20</td>
<td>20 – 22</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>9 – 11</td>
<td>34</td>
<td>14</td>
<td>14</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Spain</td>
<td>7</td>
<td>9 – 11</td>
<td>31</td>
<td>14</td>
<td>18 – 20</td>
<td>20 – 22</td>
<td></td>
</tr>
<tr>
<td>(-3)</td>
<td>17 – 19</td>
<td>21 – 28</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>(-3)</td>
<td>17 – 19</td>
<td>28 – 35</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>(-3)</td>
<td>17 – 19</td>
<td>21 – 28</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>(-3)</td>
<td>17 – 19</td>
<td>19 – 21</td>
<td>42</td>
<td>42</td>
<td>49 – 56</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>14 – 16</td>
<td>21 – 25</td>
<td>14</td>
<td>14</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>14 – 16</td>
<td>22</td>
<td>14</td>
<td>14</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>16 – 18</td>
<td>25 – 28</td>
<td>14</td>
<td>14</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>14 – 16</td>
<td>19 – 21</td>
<td>14</td>
<td>14</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Italy</td>
<td>7</td>
<td>14 – 16</td>
<td>18 – 21</td>
<td>14</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>42</td>
<td>42</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>12 – 14</td>
<td>20 – 22</td>
<td>21</td>
<td>21</td>
<td>27 – 29</td>
<td>29 – 31</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>12 – 14</td>
<td>22 – 24</td>
<td>21</td>
<td>21</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>12 – 14</td>
<td>22 – 24</td>
<td>21</td>
<td>21</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>10 – 12</td>
<td>14 – 16</td>
<td>21</td>
<td>21</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>12 – 14</td>
<td>18 – 20</td>
<td>21</td>
<td>21</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Spain</td>
<td>(-3)</td>
<td>16 – 18</td>
<td>18 – 20</td>
<td>18</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>(-3)</td>
<td>18 – 20</td>
<td>22 – 24</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>10 – 12</td>
<td>14 – 16</td>
<td>21</td>
<td>21</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>17 – 19</td>
<td>28 – 31</td>
<td>21</td>
<td>21</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>17 – 19</td>
<td>28 – 31</td>
<td>21</td>
<td>21</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>17 – 19</td>
<td>26 – 28</td>
<td>21</td>
<td>21</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Italy</td>
<td>10</td>
<td>19 – 21</td>
<td>31 – 35</td>
<td>21</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>19 – 21</td>
<td>28 – 31</td>
<td>21</td>
<td>21</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Italy</td>
<td>10</td>
<td>28 – 31</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>38 – 46</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>(-8)</td>
<td>26 – 28</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>26 – 28</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>31 – 35</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>
E2 vaccines reduce transmission

For vaccine B (Table 3.7), $\beta$ did not change from week to week after vaccination either, so $\beta$ was ultimately estimated at 0.34 (95% CI: 0.23 – 0.49). This differed significantly from the non-vaccination $\beta$ and was almost equal to the $\beta$ with vaccine A. The course of $\alpha$ looked similar for both vaccines as well. Unlike the trials with vaccine A, the trials with vaccine B did contain enough data for the time after the fourth week, from which a further increase of $\alpha$ was not observed. After pooling the last two time intervals, the $\alpha$ after 21 days was estimated at 0.82 (95% CI: 0.51 – 1.3), which was significantly different from the non-vaccination $\alpha$, and also from the $\alpha$ with vaccine A. The resulting $R_0$ was 0.41 (95% CI: 0.22 – 0.75), which was significantly different from the unvaccinated group C, and also from vaccine A. Although the two vaccines differed with respect to $\alpha$ and $R_0$, they both let $R_0$ significantly decrease to a value below 1.

3.3.3. Vaccination and outbreak size

The outbreak simulations in a non-vaccinated population resulted in either small or large outbreaks: 90 of the 1000 simulations resulted in a final size of 1 or 2 infected animals, whereas in 910 simulations the final size was 99 or 100. For the vaccinated groups there was no distinction between small and large outbreaks possible: the median and 95% confidence intervals are shown in Table 3.8.

Caption to Table 3.3.

- $^a$ Days since vaccination
- $^b$ Single positive numbers are challenge times; negative numbers between brackets are the latent periods, to be subtracted from the start of the infectious period to obtain the infection times; double numbers are the intervals in which the seropositivity started; dashes are for uninfected animals
- $^c$ Double numbers are the intervals in which the infectious periods started; dashes are for animals that were not infectious
- $^d$ Single numbers are the times of death and hence the ends of the infectiousness; double numbers are the intervals in which the infectious period ended
- $^e$ This animal was viraemic at day 11, just like the challenged animals, and has therefore been treated as a challenged animal
- $^f$ This animal was dead before the start of the experiment
- $^g$ This animal died at day 37
- $^h$ These animals had for at least one day only one virus-positive well of six replicates, which was considered a false-positive
- $^i$ This infectious period was based on fever ($T > 40 \degree C$) and was necessary to explain the two contact infections

For vaccine B (Table 3.7), $\beta$ did not change from week to week after vaccination either, so $\beta$ was ultimately estimated at 0.34 (95% CI: 0.23 – 0.49). This differed significantly from the non-vaccination $\beta$ and was almost equal to the $\beta$ with vaccine A. The course of $\alpha$ looked similar for both vaccines as well. Unlike the trials with vaccine A, the trials with vaccine B did contain enough data for the time after the fourth week, from which a further increase of $\alpha$ was not observed. After pooling the last two time intervals, the $\alpha$ after 21 days was estimated at 0.82 (95% CI: 0.51 – 1.3), which was significantly different from the non-vaccination $\alpha$, and also from the $\alpha$ with vaccine A. The resulting $R_0$ was 0.41 (95% CI: 0.22 – 0.75), which was significantly different from the unvaccinated group C, and also from vaccine A. Although the two vaccines differed with respect to $\alpha$ and $R_0$, they both let $R_0$ significantly decrease to a value below 1.

3.3.3. Vaccination and outbreak size

The outbreak simulations in a non-vaccinated population resulted in either small or large outbreaks: 90 of the 1000 simulations resulted in a final size of 1 or 2 infected animals, whereas in 910 simulations the final size was 99 or 100. For the vaccinated groups there was no distinction between small and large outbreaks possible: the median and 95% confidence intervals are shown in Table 3.8.
Table 3.4. Reconstruction of the transmission trials of group B.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Time of infection (^b) (days)</th>
<th>Start of infectious period (^c) (days)</th>
<th>End of infectious period (^d) (days)</th>
<th>Trial</th>
<th>Time of infection (^b) (days)</th>
<th>Start of infectious period (^c) (days)</th>
<th>End of infectious period (^d) (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spain – B7</td>
<td>7 – 11</td>
<td>20</td>
<td>24 – 30</td>
<td>14</td>
<td>18 – 20</td>
<td>20 – 22</td>
<td></td>
</tr>
<tr>
<td>Denmark – B14</td>
<td>(-3)</td>
<td>17 – 19</td>
<td>28 – 35</td>
<td>(-5)</td>
<td>26 – 28</td>
<td>28 – 35</td>
<td>42 – 49</td>
</tr>
<tr>
<td>Italy – B7</td>
<td>7 – 11</td>
<td>20</td>
<td>24 – 30</td>
<td>14</td>
<td>18 – 20</td>
<td>20 – 22</td>
<td></td>
</tr>
<tr>
<td>Spain – B10</td>
<td>(-3)</td>
<td>18 – 20</td>
<td>24 – 31</td>
<td>28</td>
<td>31 – 38</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Italy – B10</td>
<td>10</td>
<td>12 – 14</td>
<td>28</td>
<td>21</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>France – B21</td>
<td>10</td>
<td>12 – 14</td>
<td>31 – 38</td>
<td>42 – 49</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>10</td>
<td>14 – 16</td>
<td>24 – 31</td>
<td>21</td>
<td>25 – 29</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>10</td>
<td>14 – 16</td>
<td>24 – 31</td>
<td>21</td>
<td>25 – 29</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>10</td>
<td>14 – 16</td>
<td>24 – 31</td>
<td>21</td>
<td>25 – 29</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>10</td>
<td>14 – 16</td>
<td>24 – 31</td>
<td>21</td>
<td>25 – 29</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>10</td>
<td>14 – 16</td>
<td>24 – 31</td>
<td>21</td>
<td>25 – 29</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>10</td>
<td>14 – 16</td>
<td>24 – 31</td>
<td>21</td>
<td>25 – 29</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>10</td>
<td>14 – 16</td>
<td>24 – 31</td>
<td>21</td>
<td>25 – 29</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>10</td>
<td>14 – 16</td>
<td>24 – 31</td>
<td>21</td>
<td>25 – 29</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>10</td>
<td>14 – 16</td>
<td>24 – 31</td>
<td>21</td>
<td>25 – 29</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>10</td>
<td>14 – 16</td>
<td>24 – 31</td>
<td>21</td>
<td>25 – 29</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>10</td>
<td>14 – 16</td>
<td>24 – 31</td>
<td>21</td>
<td>25 – 29</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>10</td>
<td>14 – 16</td>
<td>24 – 31</td>
<td>21</td>
<td>25 – 29</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>10</td>
<td>14 – 16</td>
<td>24 – 31</td>
<td>21</td>
<td>25 – 29</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>10</td>
<td>14 – 16</td>
<td>24 – 31</td>
<td>21</td>
<td>25 – 29</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>10</td>
<td>14 – 16</td>
<td>24 – 31</td>
<td>21</td>
<td>25 – 29</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>10</td>
<td>14 – 16</td>
<td>24 – 31</td>
<td>21</td>
<td>25 – 29</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>10</td>
<td>14 – 16</td>
<td>24 – 31</td>
<td>21</td>
<td>25 – 29</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>10</td>
<td>14 – 16</td>
<td>24 – 31</td>
<td>21</td>
<td>25 – 29</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>10</td>
<td>14 – 16</td>
<td>24 – 31</td>
<td>21</td>
<td>25 – 29</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>10</td>
<td>14 – 16</td>
<td>24 – 31</td>
<td>21</td>
<td>25 – 29</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>10</td>
<td>14 – 16</td>
<td>24 – 31</td>
<td>21</td>
<td>25 – 29</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>10</td>
<td>14 – 16</td>
<td>24 – 31</td>
<td>21</td>
<td>25 – 29</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>10</td>
<td>14 – 16</td>
<td>24 – 31</td>
<td>21</td>
<td>25 – 29</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>10</td>
<td>14 – 16</td>
<td>24 – 31</td>
<td>21</td>
<td>25 – 29</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>10</td>
<td>14 – 16</td>
<td>24 – 31</td>
<td>21</td>
<td>25 – 29</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>10</td>
<td>14 – 16</td>
<td>24 – 31</td>
<td>21</td>
<td>25 – 29</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>
When CSFV enters a pig unit 21 days after vaccination, the number of infected pigs will remain very small with either vaccine. In most cases, only the initially infected pig will be infected and sometimes, especially with vaccine B, a few more will. Also when CSFV enters a pig unit only a week after vaccination, when $\alpha$ is still increasing for another two weeks, the size of the outbreak will be considerably reduced. With vaccine A, the upper 95% limit of the 1000 simulations reveals that only 21 out of 100 pigs will be infected, so there is only a 2.5% chance that this will be more. With vaccine B, the upper limit is 41 infected animals.

### 3.4. Discussion

This paper described an analysis of CSFV transmission trials that were carried out in the EU to test two E2 subunit marker vaccines. With the final size method, the reduction of CSFV transmission was tested. Virus transmission was significantly reduced when virus was introduced 7, 14 or 21 days after vaccination with vaccine A (produced by Bayer, Germany). Transmission was also reduced 21 days after vaccination with vaccine B (produced by Intervet, The Netherlands). The entire course method was used to estimate the parameters $\beta$, $\alpha$, and $R_0$ of the SIR model. Vaccination with both vaccines reduced the transmission parameter $\beta$ by about 50%. More importantly, the vaccines increased the recovery parameter $\alpha$, vaccine A even more than vaccine B. Both vaccines reduced $R_0$ to a value significantly below 1.
The estimated $\beta$ and $\alpha$ were used in simulations of outbreaks in a pig unit of 100 pigs. It appeared that the size of an outbreak will be greatly reduced if the pigs are vaccinated. Already when virus enters the unit only 7 days after vaccination, the number of animals that become infected is considerably lower than in the unvaccinated group.

With the final size method, the implicit assumption is made that $R_0$, and thus $\beta$ and $\alpha$, are constant over time. This was probably not the case in at least some of the

<table>
<thead>
<tr>
<th>Trial</th>
<th>Time of infection$^b$ (days)$^a$</th>
<th>Start of infectious period$^c$ (days)$^a$</th>
<th>End of infectious period$^d$ (days)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2 – 4</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>4 – 6</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>4 – 6</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>4 – 6</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>4 – 6</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>(-5)</td>
<td>12 – 15</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>(-5)</td>
<td>12 – 15</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>(-5)</td>
<td>12 – 15</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>(-5)</td>
<td>12 – 15</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>(-5)</td>
<td>12 – 15</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>(-5)</td>
<td>14 – 21</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>4 – 6</td>
<td>12 – 14$^f$</td>
<td></td>
</tr>
<tr>
<td>(-5)</td>
<td>14 – 21</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>4 – 6</td>
<td>31$^g$</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>4 – 6</td>
<td>43$^h$</td>
<td></td>
</tr>
<tr>
<td>(-5)</td>
<td>14 – 21</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>(-5)</td>
<td>14 – 21</td>
<td>28 – 35</td>
<td></td>
</tr>
<tr>
<td>(-5)</td>
<td>10 – 12</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>(-5)</td>
<td>12 – 14</td>
<td>21 – 28</td>
<td></td>
</tr>
<tr>
<td>(-5)</td>
<td>10 – 12</td>
<td>28 – 35$^f$</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>6 – 8</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>(-5)</td>
<td>12 – 14</td>
<td>14 – 16</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>4 – 6</td>
<td>8 – 10</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>4 – 6</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2 – 4$^g$</td>
<td>14 – 16$^g$</td>
<td></td>
</tr>
<tr>
<td>(-5)</td>
<td>8 – 10$^g$</td>
<td>17 – 21$^g$</td>
<td></td>
</tr>
<tr>
<td>(-5)</td>
<td>12 – 14</td>
<td>14 – 16</td>
<td></td>
</tr>
<tr>
<td>(-5)</td>
<td>12 – 14</td>
<td>14 – 16</td>
<td></td>
</tr>
<tr>
<td>(-5)</td>
<td>12 – 14</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>(-5)</td>
<td>12 – 14</td>
<td>17 – 21</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Days since start of the transmission trial 
\(^b\) Single positive numbers are challenge times; negative numbers between brackets are the latent periods, to be subtracted from the start of the infectious period to obtain the infection times 
\(^c\) The intervals in which the infectious periods started 
\(^d\) Single numbers are the times of death and hence the ends of the infectiousness; double numbers are the intervals in which the infectious periods ended 
\(^e\) Because this pig was only viraemic at the time of the other contact pigs, it was assumed that the inoculation had been unsuccessful and the pig was regarded as a contact pig 
\(^f\) These animals had for at least one day only one virus-positive well of six replicates, which was considered a false-positive 
\(^g\) Within a series of positive samples, these animals had at least one negative sample, which was considered false-negative 
\(^h\) The pig was still infectious, but the experiment stopped at this time.
Table 3.6. Estimates and 95% confidence intervals of $\beta$, $\alpha$, and $R_0$ without vaccination and with time after vaccination with vaccine A.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Day 7 – 14</th>
<th>Day 14 – 21</th>
<th>Day 21 – 28</th>
<th>Day 28 – $\infty$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta$ per interval $^a$</td>
<td>0.65</td>
<td>0.081</td>
<td>0.42</td>
<td>0.16</td>
<td>1.2</td>
</tr>
<tr>
<td>after joining $^b$</td>
<td>0.65</td>
<td>0.04</td>
<td>0.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\alpha$ per interval $^a$</td>
<td>0.065</td>
<td>0.098</td>
<td>0.50</td>
<td>5.5</td>
<td>$\infty$ $^d$</td>
</tr>
<tr>
<td>after joining $^b$</td>
<td>0.065</td>
<td>0.098</td>
<td>0.50</td>
<td>6.1</td>
<td></td>
</tr>
<tr>
<td>$R_0$ after joining $^c$</td>
<td>9.9</td>
<td>ND $^e$</td>
<td>ND $^e$</td>
<td>0.047</td>
<td></td>
</tr>
</tbody>
</table>

- Estimates without vaccination and per week after vaccination
- Estimates without vaccination and per period after vaccination within which the estimates for the subsequent weeks did not differ significantly
- Estimates without vaccination and with maximum protection by the vaccine
- Only one animal of the Denmark-A21 trial was, in some of the 100 exact reconstructions, part of the interval and had an infectious period of 0 days.
- Not determined, for the vaccine had not reached maximum protection yet.

Table 3.7. Estimates and 95% confidence intervals of $\beta$, $\alpha$, and $R_0$ without vaccination and with time after vaccination with vaccine B.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Day 7 – 14</th>
<th>Day 14 – 21</th>
<th>Day 21 – 28</th>
<th>Day 28 – $\infty$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta$ per interval $^a$</td>
<td>0.65</td>
<td>0.25</td>
<td>0.35</td>
<td>0.26</td>
<td>0.50</td>
</tr>
<tr>
<td>after joining $^b$</td>
<td>0.65</td>
<td>0.34</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\alpha$ per interval $^a$</td>
<td>0.065</td>
<td>0.069</td>
<td>0.28</td>
<td>0.86</td>
<td>0.74</td>
</tr>
<tr>
<td>after joining $^b$</td>
<td>0.065</td>
<td>0.069</td>
<td>0.28</td>
<td>0.82</td>
<td></td>
</tr>
<tr>
<td>$R_0$ after joining $^c$</td>
<td>9.9</td>
<td>ND $^d$</td>
<td>ND $^d$</td>
<td>0.41</td>
<td></td>
</tr>
</tbody>
</table>

- Estimates without vaccination and per week after vaccination
- Estimates without vaccination and per period after vaccination within which the estimates for the subsequent weeks did not differ significantly
- Estimates without vaccination and with maximum protection by the vaccine
- Not determined, for the vaccine had not reached maximum protection yet.
EU transmission trials, which makes it important to discuss the meaning of the obtained $P$-values. A $P$-value larger than 0.05 (groups A10, B7, B10, and B14) would usually mean that virus transmission was not significantly reduced in the regarded groups. Because it is known from groups A21 and B21 that transmission is significantly reduced after 21 days, most of the transmission in groups A10, B7, B10, and B14 must have taken place in the initial stage of the trials. Thus, if $P > 0.05$, the vaccine is not shown to be effective after the regarded vaccination-challenge interval. On the other hand, if the $P$-value is smaller than 0.05 (groups A7, A14, A21, and B21), it is not certain that the vaccine reduced transmission immediately after the regarded interval. It can only be concluded that the vaccine was effective soon enough to prevent much virus transmission. In group A7, protection was probably insufficient in the first days, because otherwise group A10 should have shown a significant reduction as well. In groups A14, A21, and B21, immediate reduction of transmission is very likely if the results of the entire course method are considered: $R_0$ was probably smaller than 1 during these trials.

A second implicit assumption was that the specificity and the sensitivity of the Erns-ELISAs were 100%, while they were 92% (sp.) and 74% (se.) for Erns ELISA A, and 71% (sp.) and 94% (se.) for Erns ELISA B (De Smit et al., 2000b; Floegel Niesmann, 2001). Due to the low specificity, observed contact infections in the vaccine groups might have been false-positive. Then, the effectiveness of the vaccines was underestimated in the final size analysis. By opposite reasons, the low sensitivity might have caused an overestimation of the vaccines’ effects, but this is not likely because of the multiple tests on each animal. The effects of imperfect tests on the results of the entire course method are more complicated. If a too lowly sensitive test misses a contact infection, $\beta$ is obviously underestimated, but so is $\alpha$, since the unobserved infected animal was not observed by viraemia either and consequently had a 0 days infectious period. Since $\beta$ is estimated by less data than $\alpha$, the estimate of $\beta$ is probably more sensitive to an incorrect test result. Hence, a

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Interval between vaccination and virus entry</th>
<th>Final size 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>No vaccine</td>
<td>--</td>
<td>a</td>
</tr>
<tr>
<td>Vaccine A</td>
<td>7 days</td>
<td>5</td>
</tr>
<tr>
<td>Vaccine A</td>
<td>21 days</td>
<td>1</td>
</tr>
<tr>
<td>Vaccine B</td>
<td>7 days</td>
<td>11</td>
</tr>
<tr>
<td>Vaccine B</td>
<td>21 days</td>
<td>1</td>
</tr>
</tbody>
</table>

* Without vaccination, either small outbreaks of 1 or 2 (90 of 1000 simulations) or large outbreaks of 99 or 100 infected animals (910 of 1000 simulations) occurred.
low sensitivity may underestimate $R_0$. However, there is no need to question the vaccines’ effectiveness, since $R_0$ will still be smaller than 1 if $\beta$ is underestimated (and unchanged by vaccination) and $\alpha$ is not.

The next step is to see whether the difference in sensitivity can explain the difference between the two vaccines, as suggested by (Uttenthal et al., 2001). Since increased sensitivity would lead to a higher $\beta$ and a higher $\alpha$ at the same time, $\beta$ and $\alpha$ should have higher estimates with vaccine B, if both vaccines are equally effective. However, $\beta$ is the same for both vaccines and $\alpha$ is higher with vaccine A instead of B, so the difference in sensitivity cannot explain the difference in $R_0$ between the two vaccines. Hence, the difference in $R_0$ between the two vaccines is probably a biological difference, although its nature remains unclear. Speculations have been made on the adjuvants and the quality or quantity of antigen per vaccination dose (Depner et al., 2001).

The reason not to analyse all trials simultaneously is that they were carried out in different laboratories. Namely, the risk of such a meta-analysis is that observed ‘treatment’ effects are not solely due to the treatment, but also to other differences. Therefore, the consequences of the laboratory differences should be considered for interpretation of the results. In the EU transmission experiment, three interpretation problems could arise:

1. Comparison of groups A7, A10, B7, and B10 to group C. The conclusions from trials A7, A10, B7, and B10 were that the vaccines were not yet sufficiently effective before 14 (final size method) or 21 (entire course method) days after vaccination. Hence, there is no risk of an observed ‘treatment’ effect not caused by the treatment.

2. Comparison of groups A7, A10, B7, and B10 to groups A14, A21, B14, and B21. The groups have not been directly compared, but used simultaneously to estimate $\beta$ and $\alpha$. However, an indirect comparison was made because the 7 and 10 days groups mainly provided data for weeks 2 and 3 after vaccination, and the 14 and 21 days groups for weeks 4 and 5. It might have led to a wrong conclusion about the course of $\beta$ over time, but will not have affected the conclusions that $R_0$ was smaller than 1 after 21 days or the actual estimates of $R_0$.

3. The use of the German group-C trial. Since it was the only trial carried out in Germany, Uttenthal et al. (2001) did not use this trial in their analysis at all. The reason to include the trial in our analysis after all was that the results were very similar to the other two trials of group C, and to all control trials of other CSFV transmission experiments as well (Chapter 4; Bouma et al., 2000; Laevens et al., 1998; Laevens et al., 1999).
The final assumption that needs attention is that viraemia is the indicator for infectiousness, which had been assumed in the analysis of other transmission experiments as well (Chapter 4; Laevens et al., 1998; Laevens et al., 1999). A problem with the assumption is that virus excretion might also occur while viraemia is not observed, though possibly at lower levels. However, that will be a problem with any assumption and is related to the assumption of constant infectiousness in the SIR model. Here, viraemia is used to indicate infectiousness, because viraemia is a sign of virus replication and it is likely that virus excretion can occur when virus is replicating within the animal.

Uttenthal et al. (2001) analysed the same experiment and used the Fischer’s exact test to show a difference between the two vaccines in the number of contact infections, especially after 14 days. Qualitative data inspection led to the conclusion that the vaccines reduced virus shedding from 14 days after vaccination, but that no complete protection was achieved. No conclusion could be made on whether the protection would be sufficient. Both analysis methods in our paper, the final size method as well as the entire course method, made use of the SIR model of virus transmission between animals. The effect of the vaccines was investigated by obtaining inference on the parameters of the SIR model, i.e. $\beta$, $\alpha$, and $R_0$, either by testing whether treatment groups differ or by directly estimating the parameters. The advantage of using the parameters of the SIR model is that it is easy to extrapolate the results to larger populations, which was shown by the simulations.

Vaccine A was also tested by Bouma et al. (2000), who showed that in SPF pigs, transmission was already significantly reduced 10 days after vaccination. After 14 days, $R_0$ was significantly smaller than 1. The later onset of protection in the EU trials might be explained by the use of conventional pigs, i.e. non-SPF pigs. Also, the heterogeneity due to the different laboratories might have reduced the power of the statistical tests and hence delayed finding a significant reduction in transmission. Vaccine B was also tested by Dewulf et al., who studied horizontal transmission between weaner pigs (Dewulf et al., 2000) and sows (Dewulf et al., 2001), but only vaccinated the contact pigs and not the challenged pigs. Their conclusion was that the vaccine did not prevent contact infection, which is in accordance with our findings that the largest effect of the vaccines lies in the increase of $\alpha$, and not in the decrease of $\beta$.

The decision to use vaccination as an emergency measure during a CSFV epidemic will depend on many factors. Policy makers will have to weigh epidemiological, economic, and ethical arguments, brought up by the European Union legislation, the pig industry, and the public opinion. Important aspect of the discussion will always be the effectiveness of a vaccination campaign. The ultimate effect of emergency vaccination should be that a CSFV epidemic will come quickly
E2 vaccines reduce transmission to an end, with less herds affected. However, it cannot be predicted directly from the results of the analyses how fast an epidemic may stop and how many herds may be infected. Extrapolation of the between-animal transmission to between-herd transmission, e.g. by means of mathematical modelling, is needed. What can be concluded, however, is that virus transmission between animals will be sufficiently reduced three weeks after vaccination with either marker vaccine. Already when virus enters a herd only one week after vaccination, the size of the outbreak will be much smaller than without vaccination. Hence, from the epidemiological point of view, emergency vaccination looks very promising.

Acknowledgements

The first author gratefully acknowledges financial support from STW (Technology Foundation), Utrecht, The Netherlands. All authors thank the EU Commission for financing the project; Bayer AG, Leverkusen, Germany and Intervet BV, Boxmeer, The Netherlands for supplying the vaccines; and ID-Lelystad, The Netherlands and Dr Bommeli AG, Bern, Switzerland for supplying the Ceditest and Chekit ELISA tests.

Appendix 3A

The distribution parameters $\mu_A$ and $\sigma_A^2$ for the ERS-ELISA A, and $\mu_B$ and $\sigma_B^2$ for ELISA B were estimated from the serological data of the challenged animals. Because the animals in group C had been tested with both ELISAs, they were used for estimation of both $\mu_A$ and $\sigma_A^2$, and $\mu_B$ and $\sigma_B^2$. Animals that had not become seropositive during the trials were omitted, because it concerns a distribution given that the animal becomes seropositive within the duration of the trials.

If the animal is tested with ERS-ELISA A, the probability that the random variable $T_{\text{seropos.}}$ falls within interval $(t_1, t_2)$ is

$$P(T_{\text{seropos.}} \in (t_1, t_2)) = \int_{t_1}^{t_2} PDF_{\mu_A, \sigma_A^2}(t) dt,$$

in which $PDF_{\mu_A, \sigma_A^2}(t)$ is the probability density function of the lognormal distribution with mean $\mu_A$ and variance $\sigma_A^2$, and $t$ is the time since challenge. Hence,
the data of all challenged and tested animals \( k \) were used to construct the log-likelihood function of \( \mu_A \) and \( \sigma_A^2 \):

\[
L(\mu_A, \sigma_A^2) = \sum_k \log \left[ \int_{t_k}^{t_{k+1}} \frac{\text{PDF}_{\mu_A, \sigma_A^2}(t)}{\text{PDF}_{\mu_A, \sigma_A^2}(t_k)} \text{d}t \right]
\]  

(3.1)

Maximising Eq. (3.1) for the dataset of E\textsuperscript{mos}-test A revealed the estimates for \( \mu_A \) and \( \sigma_A^2 \). Estimates for \( \mu_B \) and \( \sigma_B^2 \) were obtained analogously.

**Appendix 3B**

**3B.1. Estimation of \( \beta \)**

The method for estimation of \( \beta \) could be used for any desired time interval of the transmission trials. Within the interval under consideration, it was assumed that \( \beta \) was a constant parameter.

For estimation of \( \beta \), a survival analysis was used. Survival analysis makes use of a hazard function \( h(t, \beta) \):

\[
h(t, \beta) = \beta \cdot i(t) = \beta \frac{\text{# infectious animals}(t)}{\text{total # animals}(t)},
\]

which represents the pressure which the susceptible animals are subject to until they are infected. Each transmission trial had a different \( h(t, \beta) \), where \( i(t) \) is the infectiousness function of the trial.

To estimate \( \beta \) in a specific time interval, each susceptible animal that had been subject to one of the \( h(t, \beta) \) within that interval made up one record. For example, if we consider the week from day 14 until day 21 with vaccine A, the dataset consisted of all animals from the trials of treatment groups A7, A10, and A14 that had still been susceptible at day 14 after vaccination. Each record \( k \) consisted of two data, \( y_k \) and \( t_k \). The \( y_k \) denoted whether the \( t_k \) was truncated: \( y_k \) was equal to 1 if animal \( k \) had been infected within the considered time interval, otherwise it was 0. The \( t_k \) was either equal to the time of infection of the animal (if \( y_k = 1 \)) or to the end of the time interval (if \( y_k = 0 \)).

If the time interval started at time \( t_{\text{start}} \), the accumulated infectiousness \( I_k \) for each animal \( k \) at time \( t_k \) was calculated as
\[ I_k = \int_{t_{k-1}}^{t_k} i_k(t) \, dt , \]

in which \( i_k(t) \) was the infectiousness function of the trial which animal \( k \) had been part of. Now the probability of observing \( (y_k, t_k) \) was

\[
P(Y_k = y_k \cap T_k = t_k) = \left( \frac{\beta \cdot i_k(t_k)}{\exp(-\beta \cdot I_k)} \right)^{y_k} \left( \exp(-\beta \cdot I_k) \right)^{1-y_k}.
\]

From this probability, the likelihood function for \( \beta \) could be constructed as follows:

\[
L(\beta) = \prod_{k=1}^{n} \left[ \left( \frac{\beta \cdot i_k(t_k)}{\exp(-\beta \cdot I_k)} \right)^{y_k} \left( \exp(-\beta \cdot I_k) \right)^{1-y_k} \right]
\]

The kernel of this likelihood is the same as it would be with a set of \( n \) observations \( y_k \), each having an independent Poisson distribution with mean \( \beta I_k \) (see Aitkin et al., 1989). Therefore, the analysis was performed with a generalised linear model (GLM), where \( y_k \) denoted the response variate, \( \log I_k \) the offset, and the model was fitted with a log LINK function and a Poisson distribution. The output was an estimate of \( \log \beta \) and its estimated variance. The GLM was programmed in Mathematica® (Wolfram, 1999).

The 100 exact reconstructions of each transmission trial resulted in 100 estimates for \( \beta \) for each considered time interval. The average of the 100 estimates was considered as the final maximum likelihood estimate. The variance of the final maximum likelihood estimator consisted of two parts, viz. the average of the 100 estimated variances and the variance of the 100 estimates (Rao, 1973):

\[
\text{var}_{\theta}(\beta(\theta)) = \text{var}_{\theta}[E(\beta(\theta|\theta))] + E_{\theta}[\text{var}(\beta(\theta|\theta))],
\]
in which $\vartheta$ represents the vector of all data (the infection times, the starting and ending times of the infectious periods, and the times of death of the animals) and $\hat{\beta}(\vartheta)$ is the estimator of $\beta$ as a function of the data $\vartheta$.

3B.2. Estimation of $\alpha$

As with the estimation of $\beta$, the method for estimating $\alpha$ can be used for any desired time interval. The $\alpha$ of the considered time interval is the recovery rate of each animal infected within the time interval. The estimation method for $\alpha$ was a survival analysis as well. The hazard function $h(t, \alpha)$ of recovery was

$$h(t, \alpha) = \alpha$$

and was therefore independent of time and transmission trial.

Each animal that became infected (not infectious) within the time interval under consideration, made up one record. Each record $k$ consisted of two data, $y_k$ and $T_k$. The $y_k$ was 1 if the animal had recovered before the end of the experiment, and was 0 otherwise. The $T_k$ was the length of the infectious period. A likelihood function could be constructed similarly to the likelihood function for $\beta$, and $\alpha$ could be estimated by use of a GLM with a log LINK function and a Poisson distribution, and with $y_k$ as the response variate and $\log T_k$ as the offset. An estimate for $\log \alpha$ was obtained, with the variance of its estimator. In Chapter 2, the same method had been used for estimation of $\alpha$.

A final maximum likelihood estimate for $\log \alpha$ was obtained by averaging the 100 estimates from all exactly reconstructed transmission trials. A variance of the estimator of $\log \alpha$ was determined similarly to that of $\log \beta$, by adding the average variance of the 100 estimates to the variance of the 100 estimates.